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Abstract

A procedure for the determination of vanadium in an inorganic matrix, such as may result from the decomposition of biological samples, is described.

The method utilizes purely instrumental thermal-neutron activation analysis preceded by separation of vanadium from interfering elements by extraction with 8-hydroxyquinoline.

Samples of human blood serum were analysed by the aid of dry-ashing as well as wet-ashing techniques. Dry ashing in porcelain crucibles introduced significant amounts of vanadium into the samples, and subsequent analysis of the porcelain glaze showed a vanadium content of ~ 2 $\mu g/cm^2$.

Analytical results from samples ashed in quartz crucibles or by wet ashing showed a mean value of 0.0046 ± 0.0008 microgram of vanadium per millilitre of human blood serum.

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Contents

1. Introduction

The presence of trace elements in the human diet has an important bearing on the health of man, and a number of elements such as copper, cobalt, zinc, iron, manganese, and molybdenum are known to be essential for the functioning of vital enzyme systems.

Vanadium has been shown to inhibit cholesterol biosynthesis, and deficiency of this element in the diet may be responsible for the high death rate from cardiovascular disease in the United States as compared with the Scandinavian countries[6].

Blood plasma or blood serum is the most probable medium of distribution in the body for a number of trace elements, and the determination of vanadium in human blood is therefore of particular interest. Levels ranging from 0.00 to almost 0.10 ppm, with a mean level of 0.04 ± 0.02, have been reported by Gofman and co-workers[2], who used X-ray emission spectroscopy to obtain a standard error of measurement of $\pm$ 0.02 ppm.

It was estimated that better precision and accuracy could be obtained by using thermal-neutron activation analysis, as described in a number of papers, most recently in an article by Yule[8].

1.1. Choice of Procedure

Trace-element determination in blood by thermal-neutron activation analysis invariably requires chemical separation because of the presence of sodium and chlorine in the matrix.

The radioisotope of vanadium formed by thermal-neutron capture is V^{52} with a half-life of 3.76 minutes, and fast radiochemical separations have to be employed in order to avoid loss of sensitivity. Radiochemical procedures are given by Fukai[1], Kaiser[3] and Livingston[4], with separation times of 10-15 minutes and chemical yields of the order of 50%. No more than four samples can be processed per hour of reactor operation, and the loss of activity during processing exceeds an order of magnitude.

Separation of vanadium from interfering elements, prior to irradiation, eliminates the problems associated with the short half-life of the radioactive isotope. The number of samples that can be run per hour of reactor operation is increased considerably, and pre-irradiation separation of a large number of samples can be performed simultaneously. At the same time two important characteristics of post-irradiation radiochemical separation are lost: the absence of a reagent-blank value and the addition of

carrier to determine the yield of the chemical separation.

At the expected levels of vanadium in blood, the saving in time for a large number of samples was found to outweigh the problems associated with the development of a pre-irradiation separation procedure.

1.2. Outline of Procedure

The method adopted for the determination of vanadium comprises three separate steps: pretreatment of the sample, chemical separation of vanadium, and determination of vanadium by neutron activation analysis.

The last two steps together form a complete method of determining leachable, inorganic vanadium, applicable to a great variety of matrices, whereas the pretreatment step requires individual evaluation according to the nature of the sample material.

Because of their more general applicability, the description of the last two steps will precede that of the pretreatment of serum samples.

2. Apparatus

A TRIGA Mark I nuclear reactor, operating at a power level of 250 kW, was used for all irradiations, the pneumatic tube system being employed to irradiate samples at a thermal-neutron flux of 4.3×10^{12} n/cm^2·sec for a predetermined period of time.

A RIDL 400-channel pulse-height analyser, with a 3" x 3" solid NaI(Tl) scintillation detector, was used for recording the γ-spectra of all samples. The samples were counted directly on top of a 1/2" polystyrene β-particle absorber placed on top of the detector. A gain setting of 15 keV per channel was used in all cases.

3. Reagents

Methyl Orange Indicator, 0.1% in distilled and de-ionized water.

Potassium Hydrogen Phthalate, 10 grams dissolved in 100 ml of distilled and de-ionized water.

Aluminon Reagent, 0.1% ammonium salt of aurintricarboxylic acid in distilled and de-ionized water.

Oxine Reagent, 10 grams of 8-hydroxyquinoline dissolved in 1000 ml of chloroform.

Glass Wool, carefully cleaned by washing with chloroform.

Nitric Acid, approx. 90%.

Nitric Acid, approx. 80%, made from nitric acid, approx. 90%, by distillation in pyrex-glass apparatus.

4. Experimental

4.1. Separation of Vanadium

Separation of microgram amounts of vanadium from biological materials has been investigated by Talvitie[7], who used 8-hydroxyquinoline in chloroform as extractant. Quantitative recovery of vanadium in the 5-50 ug range was demonstrated, but normal vanadium levels in blood could not be detected by the spectrophotometric technique employed.

Using the same extraction method, we developed a modified procedure for obtaining vanadium in a matrix which does not seriously interfere with a purely instrumental determination of vanadium by neutron activation analysis.

The main interfering element, aluminium, is almost invariably present as an impurity in analytical reagents, and particularly in 8-hydroxyquinoline. It was found that extraction of aluminium into the organic phase could be prevented by the addition of aluminon, the ammonium salt of aurintricarboxylic acid, without interference with the extraction of vanadium.

The chloroform phase is not suitable for the determination of vanadium by neutron activation analysis, but 8-hydroxyquinoline was found to have sufficient solubility in pyridine - approx. 0.5 g/ml - to permit quantitative transfer into this medium after elimination of the chloroform.

The pretreated sample is digested with 1 ml of concentrated nitric acid on a hot plate at 200°F for 10 minutes, in order to bring vanadium into solution as pentavalent vanadium[x].

The solution is transferred to a 50 ml centrifuge tube by means of distilled and de-ionized water. Two drops of methyl orange indicator are added, and the solution is neutralized by the addition of 4 N ammonium hydroxide and finally adjusted at pH ~ 4.5, as visualized in the intermediate orange colour of the indicator, by means of potassium hydrogen phthalate.

After the addition of 1 ml of aluminon reagent the volume is brought to 10 ml with distilled and de-ionized water, and the solution is extracted twice with 10 ml portions of oxine reagent. The phases are mixed by vigorous stirring for not less than 30 seconds, and separation is accomplished when the solution is left standing for a short time.

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[x] Oxidation by nitric acid is 99% complete.

The separated chloroform phases are filtered through glass wool into a 50 ml beaker, combined, and left to stand in air until evaporating to dryness. The residue is dried at 115°C for 15 minutes, which causes the oxine to melt and give off the last traces of chloroform.

After cooling, the residue is dissolved in 0.5 ml of pyridine and transferred to a half-dram polyvial by means of additional pyridine. The vial is completely filled with pyridine and heat-sealed.

4.2. Instrumental Determination of Vanadium

Vanadium is determined in the oxine-pyridine matrix by irradiating for a short time in a nuclear reactor and measuring the 1.44 MeV γ-ray emitted by the thermal-neutron capture product of vanadium, vanadium-52, with a half-life of 3.76 minutes.

Figure 1 shows the γ-spectrum of a vanadium blank, irradiated for 1 minute in the pneumatic tube system of a TRIGA Mark I reactor at a flux of 4.3×10^{12} thermal neutrons/$cm^2 \cdot$ sec. The spectrum was obtained by counting the sample for 1 minute, beginning 1 minute after the end of the irradiation, on a 3" by 3" sodium-iodide scintillation detector coupled to a 200-channel pulse-height analyser.

By following the decay of the sample a number of radioisotopes could be identified; they are shown in table I, together with their half-lives and main γ-ray energies, ordered according to decreasing prominence in the spectrum.

Careful selection of counting and irradiation conditions to optimize the sensitivity and specificity of the vanadium determination without sacrificing the efficient utilization of reactor operation time, led to the establishment of a scheme consisting in 2 minutes of irradiation in the pneumatic tube system, followed by two counting periods of 1 minute live time, beginning 3 minutes and 10.5 minutes after the end of the irradiation.

The half-life discrimination attained by using the resulting $7\frac{1}{2}$ minute difference spectrum, as compared with the spectrum in figure 1, for the estimation of vanadium-52, is shown in figure 2. The actual spectrum of a vanadium blank obtained by this method is presented in figure 3 and shows the absence of peaks at 1.29 MeV and 2.75 MeV from argon-41 and sodium-24.

The energy range selected for the determination of the vanadium-52 photopeak at 1.44 MeV is restricted by the 1.28 MeV photopeak of aluminium-29 and by the aluminium-28 Compton edge at 1.555 MeV, since a too steep variation of the count rate at the upper or lower limit of the energy range would tend to invalidate the blank in case of even minor variations in gain or base life shifts from sample to sample in the multichannel analyser.

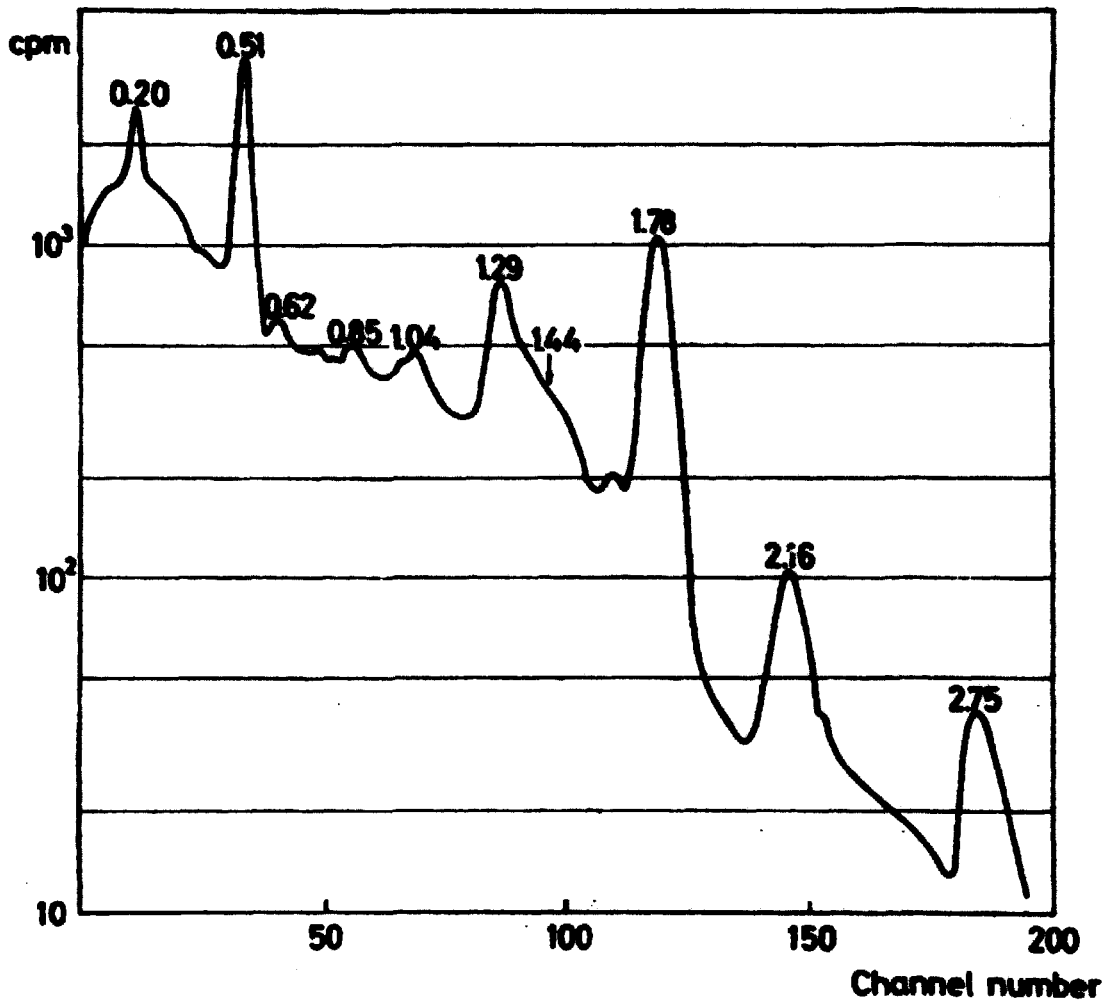

Fig. 1. γ-spectrum of a vanadium blank counted for 1 minute, beginning 1 minute after the end of a 1-minute irradiation at a thermal-neutron flux of 4.3×10^{12} n/cm^2 · sec, using a 3" x 3" NaI(Tl) scintillation detector coupled to a 200-channel analyser set at 15 keV per channel.

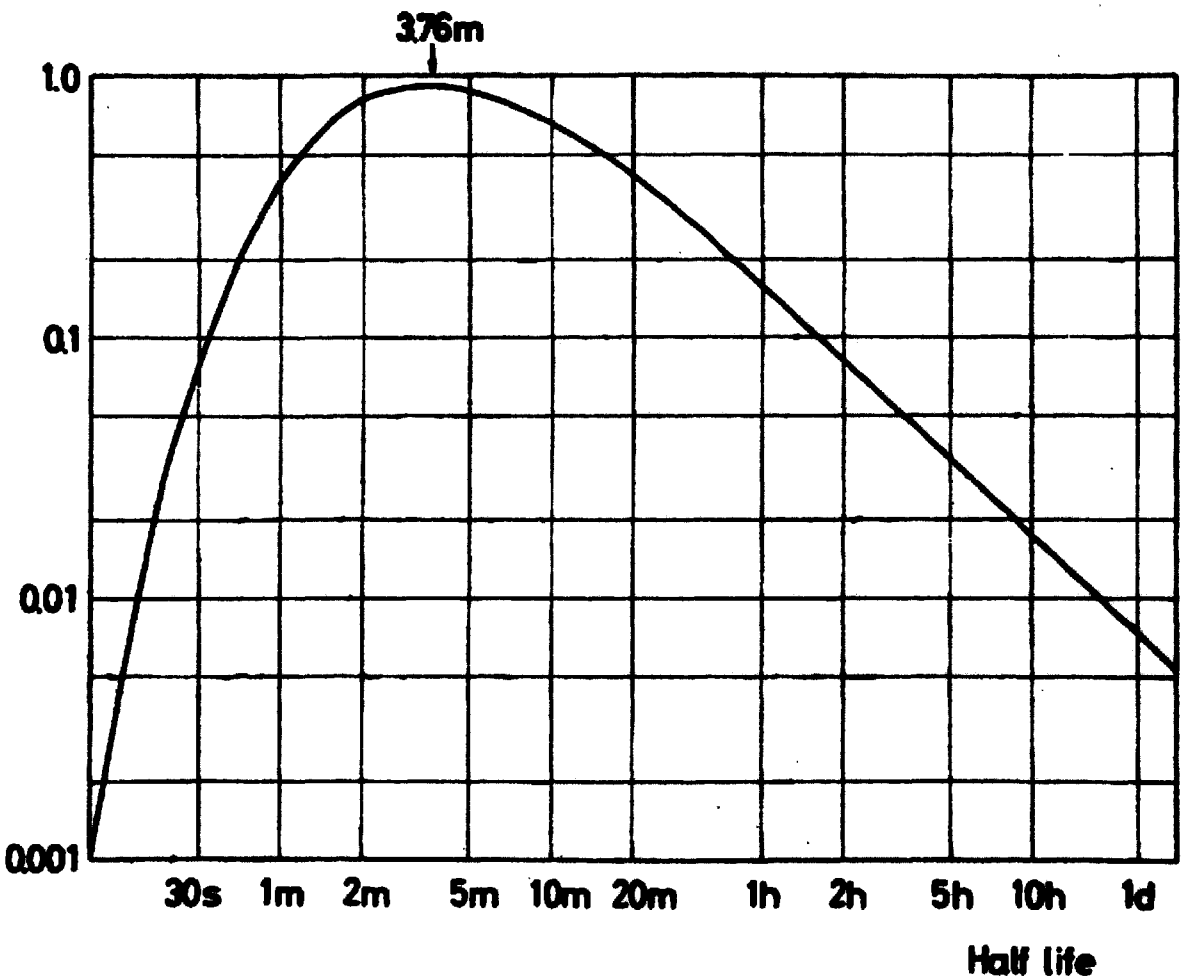

Fig. 2. Calculated reduction of contribution to γ-spectrum as a function of half-life, achieved by using the difference between spectra recorded 3 and 10.5 minutes after the end of a 2-minute irradiation, instead of using spectrum recorded 1 minute after the end of a 1-minute irradiation.

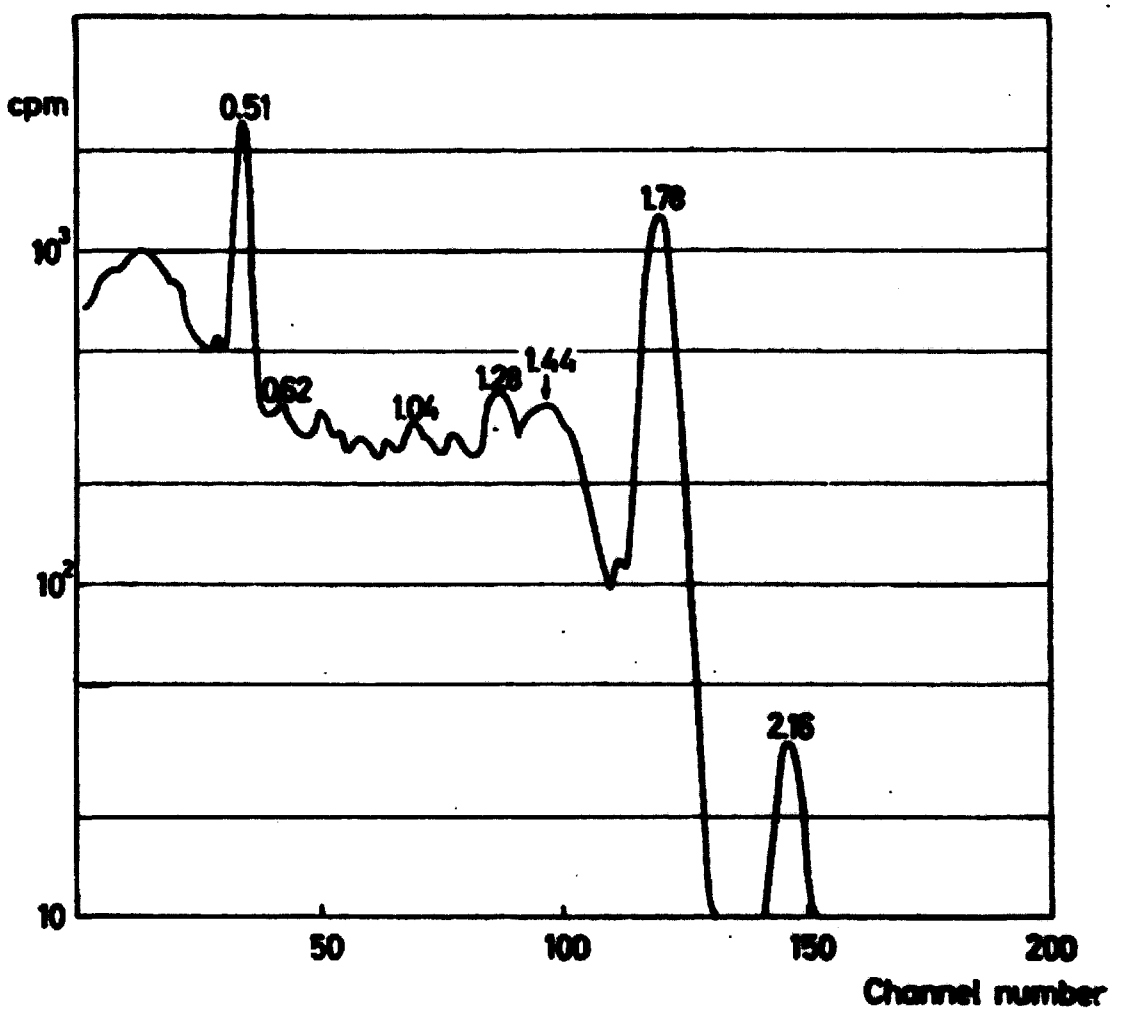

Fig. 3. Y-spectrum of a vanadium blank, representing the difference between two 1-minute counts, beginning at 3 and 10.5 minutes after the end of a 2-minute irradiation at a thermal-neutron flux of 4.3×10^{12} $n/cm^2 \cdot sec$, using a 3" x 3" NaI(Tl) scintillation detector coupled to a 200-channel analyser set at 15 keV per channel.

Table I

Components of γ-spectrum of V-blank

Radioisotope	Half-life	γ-ray energies MeV	Origin		
			Matrix constituent	Trace element in matrix	Polyvial
Oxygen-19	29 seconds	0.2, 1.366	x		
Nitrogen-13	10.0 minutes	0.51	x		
Aluminium-28	2.3 minutes	1.78		x	x
Argon-41	1.83 hours	1.29		x	x
Chlorine-38	37.3 minutes	1.64, 2.16		x	x
Sodium-24	15 hours	1.368, 2.75		x	x
Bromine-80	18 minutes	0.51, 0.62		x	
Manganese-56	2.58 hours	0.845, 1.81 2.12		x	x
Copper-66	5.1 minutes	1.04		x	
Iodine-128	25 minutes	0.45, 0.52, 0.74		x	
Vanadium-52	3.76 minutes	1.44		x	
Aluminium-29	6.6 minutes	1.28, 2.03, 2.43			x
Molybdenum-101	14.6 minutes	0.19, 0.59, 0.70, 1.02, 2.03		(x)	
Nickel-65	2.56 hours	0.37, 1.11, 1.49		(x)	

The adopted energy range of 75 keV on either side of the 1.44 MeV photopeak represents 10 channels at the usual multichannel analyser setting of 15 keV/channel and has a variation in blank value of less than one standard deviation for a $\pm$ 1/2 channel shift, while still accomodating more than 95% of the vanadium-52 photopeak counts when a sodium-iodide scintillation detector of good resolution is used.

The only identified radioisotopes contributing to the count rate in the selected energy range, apart form vanadium-52, are aluminium-28, aluminium-29, chlorine-38, and perhaps molybdenum-101. Since they all originate from trace elements in the matrix or the polyvial, their abundance is likely to vary from sample to sample, as is actually found to be the case.

The contributions from aluminium-29 and molybdenum-101 are so small, however, that even large variations in abundance would not significantly change the blank, whereas the influence of variations in aluminium-28 and chlorine-38 has to be eliminated by stripping the difference spectra of the individual samples by means of standard spectra of the two isotopes. The stripping is carried on until the 1.78 MeV and 2.16 MeV photopeaks disappear.

The sensitivity of the vanadium determination can be expressed as the standard deviation pertaining to the determination of the absence of vanadium in a sample, which is obtained by subtracting two vanadium blanks. The blanks are determined by subtracting the two spectra recorded at $7\frac{1}{2}$ minutes' interval, and the standard deviation of a blank is the square-root of the sum of the counts recorded in the selected energy range.

Transfer of the irradiated sample to an unirradiated polyvial before counting eliminates aluminium-29 and reduces aluminium-28 along with other radioisotopes originating in the polyvial. This reduces the blank value and gives some improvement in sensitivity, as shown in table II.

The transfer of an irradiated sample introduces two possible sources of error, the reproducibility of the transfer operation itself, and the reduction of the apparent half-life of argon-41 in the sample resulting from the exchange between dissolved argon-41 and inactive argon in the air.

The reduction in argon-41 activity brought about by transferring the sample exceeded a factor of 5, and the apparent half-life of argon-41 in the sample between the two counting periods was found to be long enough ($\sim$ 30 minutes) to ensure at least the same insignificant contribution to the count rate in the selected energy interval of the difference spectrum.

The variation in transfer efficiency was tested by means of added vanadium-48 tracer and was shown not to introduce any significant error,

as seen in table III.

The sensitivities indicated in table II do not take into account the effect of the stripping operation used to eliminate the influence of variations in the amounts of aluminium-28 and chlorine-38. The standard deviations in actual samples are therefore slightly larger than would be expected from the quoted sensitivities.

Table II

Average sensitivities and blank values in vanadium determination

Sample	Mean blank value µg V	Standard deviation of blank µg V	Sensitivity µg V
Not transferred	$0.009 \pm 10\%$	0.0023	0.0032
Transferred	$0.006 \pm 15\%$	0.0016	0.0023

Table III

Recovery of added vanadium

Procedure	Added vanadium µg	Recovery, %	Determined by
Complete procedure	1	96.9 ± 0.7	Activation analysis
Separation procedure	0.03	97.5 ± 0.6	Vanadium-48 tracer
Transfer operation	0.03	96 ± 1	Vanadium-48 tracer

The half-dram polyvial containing the vanadium 8-hydroxyquinoline complex in pyridine solution is irradiated for 2 minutes in the pneumatic tube system in a TRIGA Mark I reactor at a thermal flux of 4.3×10^{12} neutrons/cm^2 · sec.

If transfer is required, the polyvial is cut open with a razor blade, and the sample is transferred to another half-dram polyvial by means of a disposable Pasteur pipette.

The polyvial containing the sample is counted on top of a 1/2" perspex absorber placed directly on a 3" by 3" solid sodium-iodide scintillation detector coupled to a 400-channel pulse-height analyser. Only 200 channels are used, at a setting of 15 keV/channel. Counting takes place for 1 minute live time, beginning 3 minutes and 10.5 minutes after pile-out;

the read-out goes to magnetic tape for time-saving reasons.

This scheme permits irradiation and counting of 20 samples per hour of reactor operation.

On completion of the counting of all samples the spectra are replayed from the magnetic tapes into the memory of the pulse-height analyser in such a manner that the first 200 channels contain the difference between the 3 minute and the 10.5 minute spectrum, while the remaining 200 channels accommodate the sum of these spectra.

The digital information contained in the 400 channels is now transferred to punched cards for processing by an IBM 7044/1602 computer.

The computer programme will assume the 1.78 MeV aluminium photopeak to be found in channel 119 and will locate the selected energy region for the determination of vanadium as well as the 2.16 MeV photopeak of chlorine-38 with reference to this point.

Corrections for aluminium-28 and chlorine-38 are calculated by evaluating the photopeak area of the 1.78 MeV peak and integrating the counts in the 2.16 MeV peak region. These corrections are subtracted from the total counts in the 10 channels representing the vanadium photopeak, and the remaining counts are converted to micrograms of vanadium by comparing with a sample containing a known amount of added vanadium.

During these operations the programme will refer to the summed spectrum in the upper 200 channels for the calculation of the standard deviation of the final result.

4.3. Vanadium Recovery

The recovery of vanadium in the analytical procedure was tested by adding a known amount of tetravalent vanadium to the pretreated sample.

In one set of experiments about 1 microgram of natural vanadium was added to a sample, and the recovery was determined by activation-analysis procedure described.

In another experiment a small amount of radioactive vanadium-48 was added to the samples, and the recovery was determined by measuring the activity found in the polyvial containing the vanadium-oxine comples. The same experiment was used to determine the transfer efficiency and its variation when samples are transferred from one polyvial to another.

Table III gives the results of these experiments.

4.4. Interfering Elements

The activation-analysis method for the determination of vanadium is subject to interference from other elements which are extracted by the 8-hydroxyquinoline under the conditions employed in the separation procedure.

These elements do not include chromium and manganese, which are the main interfering elements in activation-analysis procedures employing post-irradiation separation, since they both produce vanadium-52 by reaction with fast neutrons.

Elements extracted along with vanadium, and whose presence in blood serum has been reported, were irradiated in aqueous solution and analysed as if they were normal samples. The resulting vanadium equivalents are presented in table IV; the values do not include any reduction of interference brought about by the vanadium separation procedure.

The only trace element in blood that will interfere with the vanadium determination is indium, whose presence in blood serum was reported by Gofman et al. [2], but has not been confirmed by other workers.

Table IV

Interfering elements

Element	Vanadium equivalent µg V/µg element	µg of element equivalent to 0.001 µg V	Average level in blood serum µg/ml
Fe	8×10^{-7}	>700	1
Ni	6×10^{-6}	> 80	< 0.06
Cu	8×10^{-4}	> 1	1
Zn	5×10^{-6}	>100	1
Zr	1.3×10^{-5}	> 50	0.1
Mo	2×10^{-4}	> 4	< 0.4
Sn	2×10^{-4}	> 3	0.04
In	0.1	~0.01	not established

4.5. Reagent Blanks

The validity of a blank depends upon the reproducible addition of identical reagents to all samples. In the determination of vanadium the permissible variation from sample to sample is limited by the vanadium content of the reagent to be added.

Determination of vanadium in a number of reagents to be used in this investigation was therefore performed by the present method, and the results are given in table V. The upper limits stated are at the 95% confidence level.

No one reagent is seen to be particularly critical; it should be noted,

however, that variations in the amount of nitric acid used in the various samples are limited, not by the vanadium in the nitric acid, but by the ammonium hydroxide used for its neutralization.

Table V

Vanadium impurity in various reagents

Reagent	Vanadium ppb	Amount of reagent containing 0.001 µg V ml
Potassium hydrogen phthalate, app. 10%	< 0.3	> 3
Chloroform, A.R.	< 0.3	> 2
Nitric acid, app. 70%	< 0.3	> 2
Pyridine, A.R.	< 0.5	> 1.5
Distilled yellow fuming nitric acid	< 0.8	> 1
Ammonium hydroxide, app. 58%	< 2	> 0.5
Sulphuric acid, 96%	< 2.4	> 0.2
Nitric acid, app. 90%	< 4	> 0.15

5. Pretreatment of Serum Samples

Transformation of organic-bound vanadium into inorganic vanadium ions can be achieved by dry or wet ashing of the sample.

Apparently, dry ashing lends itself most readily to pre-irradiation decomposition since no reagents are added that might swamp the original trace-element constitution of the sample. However, both methods were studied in order to evaluate their feasibility in vanadium determinations.

5.1. Dry Ashing

The loss of trace quantities of a number of elements was studied by Pijck et al.[5], who used ashing in porcelain crucibles at temperatures up to 900°C. On the basis of their findings, a 15 hour ashing at 475°C, followed by a gradual rise in temperature to 700°C over a period of 3 hours,

is expected to give little or no loss of vanadium, while still ensuring complete ashing.

This procedure was therefore tested by the addition of 1 µg of tetravalent vanadium to a sample of blood serum, and the recovery was determined by the activation-analysis method described.

Since organic-bound vanadium might conceivably have a greater volatility than inorganic, one further test was made in which an oil sample containing 2 ppm of vanadium as the porphyrin complex was used. Prior to destruction, the sample was analysed by purely instrumental activation analysis, and the recovery after destruction was determined by the present method.

The results, given in table VI, are indistinguishable from those given in table III, which shows that no loss was incurred by volatilization during the dry-ashing procedure.

Table VI

Recovery of vanadium by wet and dry ashing

Method	Vanadium present as	Recovery %
Dry ashing	tetravalent vanadium	98.2 ± 0.8
	porphyrin complex	97.7 ± 3.3
Wet ashing	vanadium-48	98.1 ± 0.8

Porcelain crucibles were used for a number of ashings; but the porcelain glaze was found to react with the alkaline ash of the serum samples, thereby introducing rather large amounts of aluminium, requiring increased addition of aluminon reagent during the vanadium separation. Furthermore, other trace elements present in the glaze might very well be introduced along with the aluminium, and they would be particularly treacherous since they would not be present in the blank.

Porcelain glaze was therefore analysed by treating a porcelain crucible with 1 ml of hydrofluoric acid, evaporating to dryness, and determining vanadium by the procedure described. A vanadium content of 8.0 ± 0.1 µg was found, corresponding to approximately 2 µg vanadium/cm^2 of porcelain

glaze.

Quartz crucibles were analysed in a similar way; they yielded only <0.03 µg of vanadium in 1 ml of hydrofluoric acid and were therefore used for the majority of the samples.

1 ml samples of blood serum are dried overnight at 95°C in a Vitreosil crucible no. 000 with cover. Ashing takes place in a muffle oven for 15 hours at 475°C, followed by a gradual increase in temperature to a maximum of 700°C during not more than 3 hours.

Along with each set of samples a blank and a standard have to be included. The standard is made up by adding an accurately known amount of vanadium, usually 1 µg, to a duplicate 1 ml serum sample.

Although to a much smaller extent than in porcelain crucibles, the serum ash also attacks the interior of the quartz crucibles. This results in partial retention of vanadium in the crucible during digestion with nitric acid and necessitates the processing of a vanadium standard along with samples to be analysed.

The variation in yield from crucible to crucible was examined with radioactive vanadium-48 and was found to be < 4%, even when the crucibles had been used for 5 consecutive ashings. However, with an increasing number of ashings the yield determined by means of vanadium-48 seems to drop faster than that determined by the recovery of an added 1 µg vanadium standard.

It is therefore not advisable to use the same crucibles more than a few times, and dry ashing is consequently not an entirely satisfactory procedure.

5.2. Wet Ashing

According to Pijck[5] no loss of vanadium should occur in wet ashing; this was confirmed by adding radioactive vanadium-48 to blood serum samles before decomposition and determining the recovery. The result is shown in table VI.

A decomposition mixture of 3:1 fuming nitric acid and concentrated sulphuric acid was found to give complete ashing in less than half an hour. Excess nitric acid was distilled off to reduce the amount of ammonium hydroxide to be used in the separation procedure.

The fuming nitric acid was found to increase the blank considerably, and a distillation of the product was needed to purify this reagent. This distillation step degrades the product to some extent, and the decomposition of the serum samples goes on much more slowly.

1 ml sample of blood serum is slowly added to a mixture of 2.5 ml of concentrated sulphuric acid and 5.0 ml of distilled fuming nitric acid in a 50 ml beaker. The solution is maintained at a temperature of about 200°F during decomposition, and more distilled fuming nitric acid is added when the solution becomes dark, accurate record being kept of the total amount of nitric acid added.

When a clear solution is obtained, the temperature is raised to boil off excess nitric acid, and after cooling the remaining sulphuric acid solution is neutralized by the addition of concentrated ammonium hydroxide.

The resulting solution is processed as described in the vanadium separation procedure, the nitric-acid digestion step being neglected.

A blank containing the same amounts of sulphuric and distilled fuming nitric acid as those used in the decomposition must be analysed along with the serum samples.

Although the decomposition of a number of serum samples is tedious, it was felt that the wet-ashing procedure was probably more satisfactory than the dry method.

6. Analytical Results

Samples of human blood serum received from the Walter Reed Hospital in Washington were analysed by dry-ashing as well as wet-ashing methods.

Samples analysed by dry ashing in porcelain crucibles invariably showed a larger vanadium content than was obtained by any of the other methods, as is clearly brought out in table VII.

The majority of samples were analysed by dry ashing in quartz crucibles; no significant difference was found between these results and those from wet ashing.

The variance of the distribution of results obtained by wet ashing and by dry ashing in quartz crucibles did not exceed that to be expected from the standard deviations of the individual determinations, and sample individualization is consequently not possible with the present method. The mean value of 36 samples in these groups is 0.0046 ± 0.0008 µg V/ml serum.

Table VII

Vanadium determination in human blood serum (55 samples)

Sample decomposed by	Mean value µg V/ml	Average standard deviation µg/ml	Standard deviation of distribution µg V/ml
Dry ashing in porcelain crucible	0.043	0.008	0.023
Dry ashing in quartz crucible	0.005	0.005	-
Wet ashing in HNO_3-H_2SO_4	0.005	0.005	-

7. Discussion

The results of vanadium determinations performed on blood serum ashed in porcelain crucibles are in excellent agreement with the findings reported by Gofman et al. [2].

The results obtained by wet ashing or dry ashing in quartz crucibles indicate that this is a mere coincidence, and that the glaze in the porcelain crucibles is the source of practically all the vanadium in these samples.

The vanadium found in samples subjected to dry ashing in quartz crucibles is not likely to have a similar origin, in the first place because of the excellent agreement with the results obtained by wet ashing, in the second place because the reduction of vanadium values is only a factor of 10 while the reduction of the vanadium content in the crucible is a factor of > 240.

No known trace elements in blood for which normal levels have been established give rise to enough interference to account for any significant fraction of the observed vanadium content. Only indium, if normally present at levels of or above 0.01 ppm, would be able to cause significant interference.

At a level of 0.01 µg in the sample to be irradiated, indium would give rise to clearly discernible peaks at 1.09 and 1.27 MeV, and since such peaks were not observed, it was concluded that the level of indium in blood is not high enough to seriously interfere with the vanadium determination.

It is therefore concluded that the observed result of 0.0046 ± 0.0008 µg vanadium per ml of serum truly represents the average vanadium content of the samples analysed.

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